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Detection of Rotavirus with a New Polyclonal Antibody Enzyme Immunoassay (Rotazyme 2) and a Commercial Latex Agglutination Text (Rotalex) Comparison with a Monoclonal Antibody Enzyme Immunoassay

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16. ABSTRACT

A total of 176 human fecal specimens were examined for the presence of rotavirus using four different assays: a monoclonal antibody enzyme immunoassay; the original polyclonal antibody enzyme immunoassay marketed by Abbott Laboratories, Chicago, IL (Rotazyme I); a modification of this assay which is now commercially available (Rotazyme II.); and a latex agglutination test (Rotalex) recently introduced by Medical Technology Corporation, Somerset, NJ. In addition, selected specimens were examined for the presence of rotavirus by electron microscopy, immune electron microscopy and by RNA gel electrophoresis. Forty specimens were positive using the monoclonal antibody enzyme immunoassay; 136 were negative. Using the results obtained with this procedure as the reference standard, the sensitivities of the Rotazyme I, Rotazyme II and Rotalex tests were 97.4, 100, and 81.6% respectively. The specificities of these three procedures were 88.8, 83.9, and 100%, respectively.

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Detection of Rotavirus with a New Polyclonal Antibody Enzyme Immunoassay (Rotazyme II) and a Commercial Latex Agglutination Test (Rotalex): Comparison with a Monoclonal Antibody Enzyme Immunoassay

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A total of 176 human fecal specimens were examined for the presence of rotavirus by four different assays: a monoclonal antibody enzyme immunoassay; the original polyclonal antibody enzyme immunoassay marketed by Abbott Laboratories, North Chicago, Ill. (Rotazyme I); a modification of this assay which is now commercially available (Rotazyme II); and a latex agglutination test (Rotalex) recently introduced by Medical Technology Corp., Somerset, N.J. In addition, selected specimens were examined for the presence of rotavirus by electron microscopy, immune electron microscopy, and RNA gel electrophoresis. A total of 40 specimens were positive in the monoclonal antibody enzyme immunoassay, and 136 were negative. Using the results obtained with this procedure as the reference standard, we found the sensitivities of the Rotazyme I, Rotazyme II, and Rotalex tests to be 97.4, 100, and 81.6%, respectively. The specificities of these three procedures were 88.8, 83.9, and 100%, respectively.

Rotavirus is now recognized as an important cause of gastroenteritis in young children (1). In addition, rotavirus has been implicated as an etiologic agent of diarrhea in older children and adults (5). Because of difficulties in propagating rotavirus in vitro (9), the laboratory diagnosis of rotavirus gastroenteritis has been based primarily on the direct detection of virus particles in clinical specimens by electron microscopy (EM) (17) or the direct detection of virus-associated antigen by any of several immunologic assays (11, 14, 15, 22). The most commonly used immunologic assay is a commercially available polyclonal antibody solid-phase enzyme immunoassay, the Rotazyme test (Abbott Laboratories, North Chicago, Ill.).

Recently, the Rotazyme test was modified by the manufacturer and reintroduced as Rotazyme II. In addition, a commercially available latex agglutination assay for rotavirus, the Rotalex test (Medical Technology Corp., Somerset, N.J.), was recently introduced. The intent of the present investigation was to examine the utility of the initial Rotazyme test (Rotazyme I), the current Rotazyme test (Rotazyme II), and the Rotalex latex agglutination test as means for detecting rotavirus in human fecal specimens. The results of these three procedures were compared with the results obtained with a monoclonal antibody enzyme immunoassay (mEIA) (10).

MATERIALS AND METHODS

Specimens. A total of 176 fecal specimens were analyzed in this study. Approximately half the specimens were from young children and had been submitted to the laboratory specifically for rotavirus determinations. The remaining specimens were chosen randomly from among unselected stool specimens submitted to the laboratory for ovum and parasite or *Clostridium difficile* analysis. All specimens were stored at −65°C before testing.

Assays. The mEIA used in this investigation has been described previously (10). Briefly, the wells of a polyvinyl chloride microtiter plate were coated with hyperimmune rabbit polyclonal antiserum prepared against a simian strain of rotavirus (SA-11). After exposure to a solution containing 1% (wt/vol) bovine serum albumin, the wells were washed three times. Fecal suspensions (10%; 0.05 ml per well) were added, incubated for 1 h at 37°C, and washed three times. A solution containing 2 μg of murine rotavirus (EDIM) monoclonal antibody per ml, prepared as described previously (4), was added (0.05 ml per well), and the plates were incubated for 1 h at 37°C. The wells were then washed three times, and bound monoclonal antibody was detected with peroxidase-labeled goat antimouse immunoglobulin G and *o*-phenylenediamine–H_2O_2 substrate. The specificity of positive results obtained with selected specimens was ascertained by demonstrating a complete lack of reactivity when preimmune rabbit serum was substituted for the rotavirus-specific rabbit polyclonal antibody capture antiserum.

Both the Rotazyme I and Rotazyme II tests were performed with commercially available reagents in accordance with recommendations of the manufacturer. Individual reactions were read with the Quantum II spectrophotometric analyzer (Abbott Laboratories). A cutoff value was defined for each assay as 0.075 units plus the value obtained with a negative control specimen. With the Rotazyme I test, specimens yielding an instrument reading of ≥125% of the cutoff value were considered positive. Specimens with values of ≤75% of the cutoff value were considered negative. Specimens with intermediate values were considered equivocal and were retested. With the Rotazyme II test, specimens with values of ≥110% of the cutoff value were considered positive, and those with values of ≤90% of the cutoff value were considered negative. As with Rotazyme I, specimens

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TABLE 1. Results obtained upon repeat testing of specimens which initially yielded equivocal results in the Rotazyme I and Rotazyme II tests

Test	Initial assay result[a]	No. of specimens tested	No. of specimens with indicated result upon retesting			
			Positive	Negative	Equivocal	
					Positive	Negative
Rotazyme I	Equivocal positive	4	0	1	1	2
	Equivocal negative	8	0	8	0	0
Rotazyme II	Equivocal positive	10	1	7	2	0
	Equivocal negative	11	0	7	0	4

[a] With the Rotazyme I test, results were defined as equivocal positive when values of 100 to 124% of the cutoff value were obtained; results were defined as equivocal negative when values of 76 to 99% of the cutoff value were obtained. With the Rotazyme II test, results were defined as equivocal positive when values of 100 to 109% of the cutoff value were obtained; results were defined as equivocal negative when values of 91 to 99% of the cutoff value were obtained.

with intermediate values were considered equivocal and were retested.

The latex agglutination assay used in this investigation, the Rotalex test, was performed with commecially available reagents in accordance with the recommendations of the manufacturer.

Selected fecal specimens were examined by EM as described by Riepenhoff-Talty et al. (18) and by immune EM (IEM) by a modification (L. J. Saif, personal communication) of the method described by Bohl et al. (2). Briefly, specimens for IEM were diluted 1:20 in phosphate-buffered saline and extracted with 1,1,2-trichloro-1,2,2-trifluoroethane, and the aqueous phase was filtered through a 0.45-μm-pore-size membrane filter. The filtrate was mixed 3:1 with hyperimmune guinea pig antiserum to simian rotavirus (SA-11), diluted 1:10 and 1:100 in buffer, and incubated for 1 h at 37°C and then for 16 h at 4°C. The suspension was centrifuged at 31,000 × *g* for 30 min, and the pellet was suspended in distilled water and centrifuged again at the same speed for an additional 30 min. The final pellet was suspended in 3% phosphoungstic acid, applied to Formvar-coated copper grids, and examined with a Philips 300 electron microscope (Philips Electronic Instruments, Inc., Mahwah, N.J.).

Selected fecal specimens were also tested for rotavirus by polyacrylamide gel electrophoresis of rotavirus RNA. The phenol-chloroform method as modified by Theil et al. (21) was used. RNA bands were detected by staining with ethidium bromide and silver (20).

Statistical analyses. Calculations of sensitivity and specificity were made as described by Galen and Gambino (7).

RESULTS

Of the 176 study specimens, 12 (6.8%) initially yielded equivocal results with the Rotazyme I test and were retested as recommended by the manufacturer. Similarly, 21 specimens (11.9%) yielded equivocal results when first tested with the Rotazyme II test, and thus the assays were repeated. The results of retesting these specimens are shown in Table 1. In all cases, the results of the second tests were used for purposes of datum analysis. Of the 176 study specimens, 2 repeatedly autoagglutinated when mixed with Rotalex reagent. These two specimens were considered uninterpretable for purposes of datum analysis.

A comparison of the results obtained with the mEIA and the Rotazyme I, Rotazyme II, and Rotalex tests is shown in Table 2. Forty specimens were positive in the mEIA. Of these, 31 were positive in each of the other three tests. Seven specimens positive in the mEIA and Rotazyme I and II tests were negative (five) or uninterpretable (two) in the Rotalex test. Six of these seven specimens were tested by EM and found to be positive. Two mEIA-positive specimens were positive in the Rotazyme II test but negative in the Rotalex test and either negative or equivocal in the Rotazyme I test. Both of these specimens were found to be negative by EM.

These last two specimens with discrepancies between the results of the mEIA and EM were further evaluated by IEM, RNA gel electrophoresis, and the mEIA by attempting to abrogate reactivity through the use of a preimmune rabbit serum as a capture antibody. Although both specimens were found to be negative by IEM, they were found to be positive by RNA gel electrophoresis. Furthermore, all reactivity was lost in the mEIA when preimmune rabbit serum was used as a capture antibody.

A total of 136 specimens were negative in both the mEIA and the Rotalex test. Of these 136 specimens, 13 were positive in both Rotazyme I and II tests, 8 were positive only in the Rotazyme II test, and 2 were positive only in the Rotazyme I test. Of the 23 specimens negative in both the mEIA and the Rotalex test but positive in either the Rotazyme I or II test, 20 were analyzed for the presence of rotavirus by EM. EM failed to reveal virus particles in all 20 specimens. The 13 specimens positive in the Rotazyme I and II tests but negative in the mEIA and the Rotalex test were also evaluated by RNA gel electrophoresis; all were negative.

Using the results of the mEIA as a reference standard and excluding from the datum analysis specimens which yielded either uninterpretable or equivocal results, the sensitivity and specificity of the Rotalex, Rotazyme I, and Rotazyme II tests were calculated to be 81.6 and 100%, 97.4 and 88.8%, and 100 and 83.9%, respectively.

DISCUSSION

The mEIA used as a reference standard in this investigation has been shown previously to be a highly sensitive and specific means for detecting the presence of rotavirus in human fecal specimens (10). In the current study, of a total of 33 specimens analyzed by both the mEIA and EM, concordance between the results of these two assays was achieved with 31 specimens. Two specimens were positive by mEIA but negative by both EM and IEM. It was felt that the results of the mEIA obtained with these two specimens represented true-positive results, however, since in both cases, RNA gel electrophoresis results were positive and all reactivity with the mEIA was abrogated when preimmune rabbit serum lacking rotavirus antibody was used as a capture antiserum.

When compared with the mEIA, both the Rotazyme I and II tests were found to have high sensitivity, i.e., 97.4 and 100%, respectively. Both tests, however, lacked specificity. The specificity of the Rotazyme I test was estimated to be 88.8%; the specificity of the Rotazyme II test was estimated to be 83.9%. It appears from these results that the Rotazyme II test is more sensitive than Rotazyme I test but also less specific.

The sensitivities of both Rotazyme tests, as determined in

TABLE 2. Comparison of the results of testing human fecal specimens for the presence of rotavirus with the mEIA, the Rotazyme I and Rotazyme II tests, the Rotalex test, and EM

Result of the mEIA	No. of specimens tested by Rotazyme I, Rotazyme II, and Rotalex tests	Result obtained with:			No. of specimens tested by EM	Results obtained with EM
		Rotazyme I test	Rotazyme II test	Rotalex test		
Positive	31	Positive	Positive	Positive	5	Positive
	7	Positive	Positive	Negative[a]	6	Positive
	2	Negative[b]	Positive	Negative	2	Negative[c]
Negative	113	Negative[b]	Negative[d]	Negative		
	13[e]	Positive	Positive	Negative	12	Negative
	8	Negative[b]	Positive	Negative	6	Negative
	2	Positive	Negative	Negative	2	Negative

[a] Two specimens in this group yielded results with the Rotalex test which were uninterpretable because of autoagglutination.
[b] One specimen in this group yielded an equivocal result in the Rotazyme I test.
[c] Both specimens were further tested by IEM, RNA gel electrophoresis, and a confirmatory mEIA. Although both specimens were found to be negative by IEM, positive results were obtained in RNA gel electrophoresis and the confirmatory mEIA.
[d] Six specimens in this group yielded equivocal results in the Rotazyme II test.
[e] These 13 specimens were also evaluated by RNA gel electrophoresis and found to be negative.

the present study, are in general agreement with the observations of others using the Rotazyme I test (3, 8, 12, 19, 23). Previous studies with the Rotazyme I test demonstrated an overall specificity of 90 to 95% (3, 8, 12, 19, 23). The specificities of both the Rotazyme I and II tests in our investigation were slightly lower. These differences might be explained by the ages of the patients from whom fecal specimens were obtained in our study. Krause et al. reported a higher-than-normal incidence of false-positive results in the Rotazyme I test for infants less than 3 months of age (13). It is possible that a number of specimens from neonates and infants in this very young age group were included in our study. The exact ages of individuals providing stool specimens in our study were, however, unknown.

Of concern with both Rotazyme procedures were the relatively large numbers of specimens which initially yielded equivocal results. According to the recommendations of the manufacturer, these specimens warrant retesting. In a clinical laboratory performing large numbers of rotavirus assays, the cost and inconvenience of such retesting could be considerable. An analysis of the results obtained upon repeat testing of initially equivocal specimens suggests that such retesting is probably not always necessary. Twelve specimens initially yielded equivocal results in the Rotazyme I test. Of these 12 specimens, 8 had assay values of 76 to 99% of the cutoff value and were defined as equivocal negative. In all eight cases, a negative result was obtained upon repeat testing. Similarly, 21 specimens were initially equivocal in the Rotazyme II test. Of these 21 specimens, 11 had values interpreted as equivocal negative, i.e., 91 to 99% of the cutoff value. In no case did repeat testing of these 11 specimens yield a positive or even an equivocal positive result. Based on these observations, specimens initially yielding equivocal negative results, as defined above, could probably be precluded from repeat testing. Such an approach would decrease by at least 50% the number of specimens which would require retesting.

The Rotalex test was highly specific (i.e., 100%) but lacked sensitivity (i.e., 81.6%). Nearly identical results were obtained in a recent evaluation of the Rotalex test in France (16).

Based on the results of our study as well as the investigations of others, it is clear that neither the Rotazyme tests nor the Rotalex test is completely satisfactory; however, it would appear that both the Rotalex and the Rotazyme II tests have utility as diagnostic tests, but in different clinical situations. The Rotalex test, because of its simplicity, lack of requirement for instrumentation, and excellent specificity, might be used to rapidly screen symptomatic ambulatory patients suspected of having rotavirus infections, perhaps directly in outpatient clinics or private physician's offices. Because the Rotalex test appears to be highly specific, a positive result could be considered definitive. The relative lack of sensitivity of the Rotalex test, however, would require that negative specimens, at least those obtained from patients strongly suspected of having rotavirus infections, be submitted to a diagnostic laboratory for analysis with a more sensitive rotavirus detection test. The Rotazyme II procedure, because of its excellent sensitivity, is better suited to an analysis of fecal specimens derived from inpatients. Because of the risk of the nosocomial spread of rotavirus within pediatric units (6), the consequences of missing a positive result can be serious. For this reason, the inevitable false-positive results which would arise when using a test like the Rotazyme II test with a relatively low specificity could be tolerated given the fact that few, if any, positive results would be missed.

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